PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

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27. OTT. 2003

Date of mailing *(day/month/year)* 16 October 2003 (16.10.03)	
Applicant's or agent's file reference PC430PR	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP03/03667 ✓	09 April 2003 (09.04.03) ✓	10 April 2002 (10.04.02) ✓

Applicant: MEDESTEA INTERNAZIONALE S.R.L. ✓

1. Notice is hereby given that the International Bureau has **communicated**, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

   AU, AZ, BY, CH, CN, CO, DE, DZ, HU, JP, KG, KP, KR, MD, MK, MZ, RU, TM, US ✓

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CR, CU, CZ, DK, DM, EA, EC, EE, EP, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PH, PL, PT, RO, SC, SD, SE, SG, SK, SL, TJ, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW ✓

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-*bis*)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 16 October 2003 (16.10.03) under No. 03/085099

4. **TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase**

   The applicable time limit for entering the national phase will, **subject to what is said in the following paragraph,** be **30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of **19 months** from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

   In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For **regular updates on the applicable time limits** (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   For filing a **demand for international preliminary examination**, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

   It is the applicant's **sole responsibility** to monitor all these time limits.

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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*
— *of inventorship (Rule 4.17(iv)) for US only*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A PROCESS FOR THE PREPARATION OF STEM CELLS FROM HUMAN MUSCLE TISSUE AND ADIPOSE TISSUE, AND STEM CELLS OBTAINABLE BY THIS PROCESS

(57) Abstract: The invention relates to a process for the preparation of the human stem cells from muscle tissue or adipose tissue. The process provides for the incubation of cells obtained from a sample of muscle tissue or adipose tissue in a medium comprising BSA, Bfgf, EGF, VEGF, LIF, heparin and usual inorganic salts, natural acids and vitamins necessary for the growth of mamalian cells. The invention relates also to the human muscle stem cells (hMSC) and human adipose tissue cells (hFSC) obtainable by this process.

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AMENDED CLAIMS

[received by the International Bureau on 14 July 2004 (14.07.04);
original claims 1 - 14 amended (4 pages)]

1. A process for the preparation of human stem cells from a sample of human adipose or muscle tissue, said human stem cells being capable of differentiating into nerve cells, vascular cells and bone cells, the process comprising the steps of:

a) preparing a cell suspension from a sample of human adipose or muscle tissue;

b) recovering the cells from the cell suspension; and

c) incubating these cells in a medium comprising BSA, bFGF, EGF, VEGF, LIF, heparin and usual inorganic salts, natural amino acids and vitamins necessary for the growth of mammalian cells.

2. A process according to claim 2, wherein the medium is DMEM/F12 supplemented with: from 0.4% to 0.8% of BSA, from 5 to 20 ng/ml of bFGF, from 10 to 40 ng/ml of EGF, from 2.5 to 10 ng/ml of VEGF, from 5 to 20 ng/ml of LIF, from 1 to 20 μg/ml of heparin, from 1.8 to 3 mg/ml of glucose, from 2 to 2.5 mg/ml of $NaHCO_3$, from $2.5x10^{-3}$ to $7.5x10^{-3}$ M of Hepes, from 50 to 200 μg/ml of apotransferrin, from 10 to 30 μg/ml of insulin, from $3x10^{-4}$ to $7x10^{-4}$ M of putrescine, from $4x10^{-8}$ to $8x10^{-8}$ M of selenium, from $1x10^{-8}$ to $3x10^{-8}$ M of progesterone.

3. A process according to claim 1 or 2, wherein the tissue sample is a human skeletal muscle sample and the stem cells are human muscle stem cells (hMSC).

4. A process according to claim 3, wherein step a) comprises the digestion of the skeletal muscle sample with trypsin.

5. A process according to claim 3 or 4, wherein step c) comprises:

c_1) resuspending the cells recovered from the cell suspension of step a) in the growth medium as defined in claim 1 or 2;

c_2) incubating the cell suspension obtained in the previous step inside a container for cell cultures, which has previously been treated with type I collagen, for from 18 to 24 hours at a temperature of approximately 37°C and in a 5% CO_2 atmosphere;

c_3) removing the growth medium from the container and replacing it with an identical freshly prepared growth medium; and

c_4) incubating for a further 48 to 72 hours, thereby obtaining the formation of small roundish cells adhering to the walls of the container, the small adherent roundish cells being human muscle stem cells (hMSC).

6. Human muscle stem cells (hMSC) capable of differentiating into nerve cells, vascular cells and bone cells, obtainable by a process according to any one of claims 3 to 5.

7. A process according to claim 1 or 2, wherein the tissue sample is a human adipose tissue sample and the stem cells are human adipose tissue stem cells (hFSC).

8. A process according to claim 7, wherein said step c) comprises:

c_1) resuspending the cells recovered from the cell suspension of step a) in the growth medium as defined in claim 1 or 2;

c_2) incubating the cell suspension obtained in the previous step inside a container for cell cultures, which has previously been treated with type I collagen, for from 18 to 24 hours at a temperature of approximately 37°C and in a 5% CO_2 atmosphere;

c_3) recovering the cells not adhering to the walls of the container and resuspending them in freshly prepared growth medium as defined in claim 1 or 2;

c_4) placing the cell suspension obtained in the previous step in a second container for cell cultures which has not previously been treated with collagen and cultivating the cells therein for from 7 to 10 days, thus obtaining the formation of floating cell aggregates, the cells of these aggregates being human adipose tissue stem cells (hFSC).

9. A process according to claim 8, wherein steps c_2) and c_3) are repeated twice more.

10. Human adipose tissue stem cells (hFSC) capable of differentiating into nerve cells, vascular cells and bone cells, obtainable by a process according to any one of claims 7 to 9.

11. Stem cells according to claim 6 or 10 for use in the regeneration of tissue selected from the group consisting of bone tissue, cartilaginous tissue, endothelial tissue, smooth muscle tissue, striated muscle tissue and nerve tissue.

12. Stem cells according to claim 6 or 10 for use in the treatment of ischaemic tissue, in the repair of vascular damage, in the cell treatment of myocardial infarct, in co-transplantation with other stem cells or tissues, in the production of growth and/or trophic factors, in the production of hormones, in tissue bioengineering, in the regeneration of peripheral nerves, in the treatment of multiple sclerosis, in the treatment of myocardial infarct, in the treatment of Alzheimer's disease or in the treatment of Parkinson's disease.

13. Use of stem cells according to claim 6 or 10 for the preparation of a medicament for the regeneration of tissue selected from the group consisting of bone tissue, cartilaginous tissue, endothelial tissue, smooth muscle tissue, striated muscle tissue and nerve tissue.

14. Use of stem cells according to claim 6 or 10 for the preparation of a medicament for the treatment of ischaemic tissue, the repair of vascular damage, the cell treatment of myocardial infarct, co-transplantation with other stem cells or tissues, the regeneration of peripheral nerves, the treatment of multiple sclerosis, the treatment of myocardial infarct, the treatment of Alzheimer's disease or the treatment of Parkinson's disease.